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1 **The impact of chronic pain on brain gene expression**

2

3 **Lily Collier[1,2], Carina Seah[3], Emily M. Hicks[3], Traumatic Stress Brain Research Group,**
4 **Paul E. Holtzheimer[4,5], John H. Krystal[2,6], Matthew J. Girgenti[2,6], Laura M. Huckins[2]*,**
5 **Keira J.A. Johnston[2]***

6

7 1. Department of Biological Sciences, Columbia University, New York City, NY
8 2. Department of Psychiatry, Division of Molecular Psychiatry, Yale University, New
9 Haven, CT
10 3. Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount
11 Sinai, New York City, NY
12 4. National Center for PTSD, U.S. Department of Veterans Affairs
13 5. Department of Psychiatry, Geisel School of Medicine at Dartmouth, Lebanon, NH 03756,
14 USA
15 6. Clinical Neuroscience Division, National Center for PTSD, VA Connecticut Healthcare
16 System, West Haven, CT
17

18 **Abstract**

19 **Background**: Chronic pain affects one fifth of American adults, contributing significant public
20 health burden. Chronic pain mechanisms can be further understood through investigating brain
21 gene expression. **Methods**: We tested differentially expressed genes (DEGs) in chronic pain,
22 migraine, lifetime fentanyl and oxymorphone use, and with chronic pain genetic risk in four
23 brain regions (dACC, DLPFC, MeA, BLA) and imputed cell type expression data from 304
24 postmortem donors. We compared findings across traits and with independent transcriptomics
25 resources, and performed gene-set enrichment. **Results**: We identified two chronic pain DEGs:
26 B4GALT and VEGFB in bulk dACC. We found over 2000 (primarily BLA microglia) chronic
27 pain cell type DEGs. Findings were enriched for mouse microglia pain genes, and for hypoxia
28 and immune response. Cross-trait DEG overlap was minimal. **Conclusions**: Chronic pain-
29 associated gene expression is heterogeneous across cell type, largely distinct from that in pain-
30 related traits, and shows BLA microglia are a key cell type.

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31 **Introduction**

32 Chronic pain affects roughly one fifth of adults in the United States [1,2], and chronic pain
33 conditions (including low back pain and headache disorders) rank in the global top 10 non-
34 infectious diseases contributing to disability adjusted life years [3]. Chronic pain is defined as pain
35 lasting at least three months [4,5]. It is associated with a wide range of physical conditions and it
36 may follow surgery, injury or physical trauma [6,7]. Mechanisms of chronic pain development are
37 not fully understood, and for many individuals treatment is not effective, particularly long term [8–
38 10].

39

40 Understanding the etiopathology of chronic pain requires that researchers investigate and
41 compare the roles of predisposition to pain development, and effects stemming from experience
42 of pain. Studies of predisposition including large genome-wide association studies have
43 uncovered hundreds of trait-associated variants and genes [11,12], and have suggested various
44 Central Nervous System (CNS) pathways to be important in chronic pain [13]. However, these
45 findings (as with GWAS findings more broadly) have yet to be translated into full mechanistic
46 understanding of disease development, or into actionable treatment. One step toward addressing
47 this knowledge gap is through analysis at the gene expression and transcriptomic level,
48 intermediate steps between genotype (GWAS findings) and phenotype (chronic pain). We and
49 others have applied transcriptomic imputation approaches to translate GWAS findings into gene-
50 tissue associations [14,15], identifying brain regions with a putative role in chronic pain including
51 hippocampus, cerebellum, amygdala and frontal cortex among others. However, we do not yet
52 know which brain regions and cell types are primarily involved with chronic pain development.
53 Rodents studies have identified >700 differentially expressed genes in brain and spinal cord,
54 associated with a range of phenotypes including chronic pain, post-surgical pain, neuropathic
55 pain, and spinal injury [16–20].

56

57 Meanwhile, human gene expression studies have focused on identifying genes associated with
58 specific chronic pain conditions, including fibromyalgia [21], osteoarthritis [22], migraine [23], sickle
59 cell disease [24], endometriosis [25], and others [26–31]. However, sample sizes tend to be small, and
60 typically do not include brain tissue. Here, we present the largest study of differentially
61 expressed genes of brain tissue in chronic pain to date. We assess gene expression in post-

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62 mortem brain tissues from 304 human donors, from four brain regions previously implicated in
63 pain processing and chronic pain (basal and medial amygdala, prefrontal and anterior cingulate
64 cortex), with detailed phenotype data. We ask whether chronic pain has region- and cell-type
65 specific transcriptomic signatures in the human brain. Defining chronic pain is complex, and it is
66 vital to differentiate pain-associated transcriptomic signal from genes associated with
67 predisposition, specific pain-causing conditions (such as migraine), or genes with expression
68 changes resulting from use of pain-alleviating medications (such as opioids). Using donor
69 phenotype and genotype data, we determine whether the transcriptomic signatures identified in
70 our analyses arise from predisposition to chronic pain, experience of pain itself, or due to
71 treatments or medications taken to alleviate pain (e.g., fentanyl, oxymorphone).
72

73 **Methods**

74 **Postmortem brain dataset description**

75 We obtained data for 304 postmortem human brains, donated at time of autopsy through US
76 medical examiner's offices, from the Veteran's Association (VA) National PTSD Brain Bank and
77 Traumatic Stress Brain Research group (**Table 1**). Retrospective clinical diagnostic review of
78 toxicology and next-of-kin interviews were performed in this previous study, and a wide range of
79 clinical, anthropometric, psychiatric and life history information, including on trauma history,
80 drug use, and presence of chronic pain, was collected. 115 total donors were female, 189 were
81 male, with a mean age of death of 46.75 years.
82

83 Bulk tissue was sampled from four regions of each brain; the dorsolateral anterior cingulate
84 cortex (dACC), dorsolateral prefrontal cortex (DLPFC), basolateral amygdala (BLA), and medial
85 amygdala (MeA), totaling 1216 samples. Collection of brain tissue samples postmortem,
86 genotyping, and producing gene expression data for this cohort is described in detail
87 elsewhere[32,33].
88

89 **Chronic pain phenotype**

90 Chronic pain in this dataset was encoded as present/absent (1/0) by researchers associated with
91 the original data collection, and may include a wide range of chronic pain conditions, locations
92 of chronic pain on the body, and varying severity. Chronic pain presence/ absence was derived

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93 from multiple data sources including health records and next of kin interview during
94 retrospective clinical diagnostic review. Migraine, fentanyl lifetime use, and oxymorphone
95 lifetime use were determined using the same approach.
96
97 **Imputation of cell-type level gene expression data from bulk tissue**
98 To obtain imputed cell type level gene expression, we carried out cell type deconvolution and
99 subsequent estimation of cell type level gene expression. Since choice of reference panel impacts
100 cell type imputation accuracy [34], we used two reference panels. We imputed both cortical bulk
101 tissues (DLPFC, dACC) with PsychENCODE reference cell types [35–37], and both amygdala bulk
102 tissues (BLA, MeA), using an amygdala specific reference panel [38] (see Supplement). Six cell
103 types were available in both reference datasets (endothelial cells, microglia, inhibitory neurons,
104 excitatory neurons, oligodendrocytes and astrocytes) (see also Supplement).
105
106 Imputation was carried out in the same way across all tissues. First, using the bulk RNA-seq raw
107 counts, we constructed a matrix of transcripts, using CIBERSORTx [39] and the appropriate
108 reference panel (cortex or amygdala) to estimate cell type proportions for bulk tissue regions.
109 bMind [40] was used to impute cell type-specific gene expression for a total of six cell types in
110 each region (astrocytes, endothelial cells, excitatory neurons, inhibitory neurons, microglia, and
111 oligodendrocytes).
112
113 **Finding differentially expressed genes using bulk tissue and cell type level expression data**
114 We calculated surrogate variables (SVs) using bulk tissue gene expression data and preserving
115 for chronic pain, in order to capture sources of variability not directly measured in the study [41]
116 using R package sva [42]. We then checked for significant correlation between these SVs and our
117 measured variables using Pearson correlation tests. We next removed variables with more than
118 half of samples showing missing data, and then retained variables that were not correlated with at
119 least one SV (representing variables that were not fully captured by SVs). We then checked for
120 collinearity amongst these retained variables, removing variables and re-checking correlations in
121 a stepwise manner until a set of non-correlated (non-collinear) variables remained. These
122 uncorrelated-with-SVs, non-collinear variables were included as covariates alongside our SVs
123 and chronic pain phenotype in a linear regression model to find differentially expressed genes in

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124 chronic pain (see Table S1 for regression model per bulk tissue/ cell type analysis). We applied
125 Bonferroni correction within-tissue (total 4 tissues) to our regression p value results. We then
126 repeated DEG analysis steps (SVA, SV correlation check, collinearity check, DEG regression
127 analysis) for cell type level gene expression data, applying Bonferroni correction within tissue
128 within cell type (total 24 sets) to our results.
129
130 We repeated DEG analyses for lifetime fentanyl use, lifetime oxymorphone use, and migraine in
131 both bulk tissue and cell type. We carried out correlation tests among all lifetime opioid use
132 variables – fentanyl was significantly ($p < 0.05$, Pearson correlation test) correlated with most
133 other lifetime opioid use variables, except oxymorphone. Therefore, we chose fentanyl and
134 oxymorphone for DEG analyses, in order to investigate whether any chronic pain DEGs we
135 found reflected opioid treatment or use as a result of chronic pain.
136

137 Multiple Test Corrections

138 Unless stated otherwise, multiple test correction was carried out in each DEG analysis using a
139 Bonferroni correction for the number of genes tested. For bulk tissue analyses we applied
140 Bonferroni correction within-tissue, and for cell type analyses we applied Bonferroni multiple
141 testing correction within-tissue within cell type. We also apply a secondary experiment-wise
142 multiple test correction (see Supplement).
143

144 Pathway analyses of chronic pain DEGs using FUMA

145 We carried out gene-set enrichment tests within FUMA [43] using significant genes ($P_{Bonferroni} <$
146 0.05). We required at least 10 unique significant genes for inclusion in this analysis. Taking
147 DEGs from cell-type analyses in each tissue (total N=24 initial lists of significant genes), we
148 included gene lists containing more than ten unique genes. We used as background 17,550 genes
149 that were included in each of our DEG analyses per cell type, available in FUMA, and assigned
150 an Ensembl gene ID.
151

152 Comparing cell type DEGs and DAM/ARM and McGill Transcriptomic Pain Signatures
153 data

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154 We next compiled a list of differentially expressed genes from the 'transcriptomics pain
155 signatures database' (TSPdb) maintained by the human pain genetics lab at McGill University [44],
156 where data is available for human whole blood, synovial fluid, and cartilage transcriptomics
157 experiments. We retained genes from human experiments in whole blood (the majority of the
158 human experiments in this database 1,260,387 (76% of experiments)), where the contrast
159 investigated was 'pain vs no pain', where sequencing was through high throughput and not
160 microarray, and where experiments had both males and females included. This resulted in a list
161 of 203,984 experimental results comprised of 23,499 unique genes, 15,968 of which are also
162 tested in each of our cell type DEG analyses. We carried out a series of Fisher's exact tests for
163 enrichment of each list of chronic pain genes per cell type in this McGill database list, with the
164 list of 15,968 shared genes as background. Note that it was not possible to directly test for DEG
165 enrichment (i.e., identical gene-tissue results) as opposed to enrichment for genes implicated in
166 DEGs (regardless of tissue), as the only human tissues available in the McGill database are non-
167 brain.
168
169 We then compiled a list of genes from the same database from experiments on mice (Mus
170 musculus) and in brain and nervous tissues (Medical Subject Headings (MeSH) beginning
171 'A08..') (2,940,318 experiments). We then again included results where contrast investigated
172 was pain vs no pain, experiments used high throughput sequencing, and including both males
173 and females (206,827 experiments). The final database subset contained results for four tissues:
174 microglia (A08.637.400), brain stem (A08.186.211.132), spinal ganglia (A08.800.350.340), and
175 sciatic nerve (A08.800.800.720.450.760). We carried out a series of Fisher's exact tests for
176 enrichment of each list of chronic pain genes per cell type in each set of database results per
177 tissue.
178
179 We also compared our chronic pain microglia DEGs to disease-associated microglia (DAM) and
180 activation response microglia (ARM) genes, using gene lists from publicly available information
181 on DAMs and ARMs from two studies [45,46] compiled by Thrupp et al [47] (Thrupp et al Table S1
182 'Genesets previous studies') (360 genes, of which 349 were also tested in our analyses). We did
183 not carry out formal enrichment tests as it was not possible to discern the total number of genes
184 shared as background between our analyses and the Thrupp et al gene list experiments.

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185

186 **Polygenic risk score analyses**

187 We carried out analyses to uncover DEGs associated with polygenic risk of (proxying
188 predisposition to) chronic pain, using GWAS summary statistics for multisite chronic pain
189 (MCP) [48].

190

191 First, using PLINK 1.9 and 2.0 [49] we carried out recommended quality control (QC) procedure
192 for PRSice2 PRS analyses [50]. We removed duplicate and ambiguous (palindromic) SNPs from
193 our GWAS summary statistics, as well as SNPs with a low minor allele frequency (MAF < 0.01)
194 and low imputation quality (< 0.8).

195

196 Next, we carried out QC steps for genotype data associated with the postmortem brain dataset.
197 We excluded SNPs with MAF < 0.01, duplicate SNPs, SNPs with missing genotype call rates >
198 0.01, and SNPs not in Hardy-Weinberg equilibrium ($p < 1x10^{-6}$). We also removed samples with
199 missing call rates greater than 0.01. Next, we LD-pruned SNPs in order to obtain a set of SNPs
200 in linkage equilibrium, using window size 200 bp, step size 25 bp, and a correlation threshold of
201 0.25 and PLINK –indep-pairwise. Then, we calculated sample heterozygosity using PLINK 2.0 –
202 het function, and excluded samples with values greater than 3 SDs from the mean sample
203 heterozygosity value. We then used PLINK 1.9 –check-sex function to flag samples where
204 genetic sex did not match reported sex, which were then removed. We filtered for genetic
205 relatedness using PLINK 1.9 –rel-cutoff at a threshold of $\pi > 0.125$ (3rd degree relatives). We
206 performed PCA analysis using PLINK pca and the QC'd genotype data to obtain the first 20
207 genetic principal components for inclusion in the PRS calculation as covariates, to account for
208 population structure (particularly differing genetic ancestry between source GWAS cohort and
209 postmortem brain tissue donors).

210

211 We then used PRSice2 to calculate MCP-PRS for these N=250 post-QC postmortem brain cohort
212 participants. PRSice2 runs multiple PRS analyses at varying GWAS p value thresholds for
213 inclusion, returning a best-fit PRS (measured by PRS-trait regression R2 value). All SNPs
214 passing QC and associated with MCP were included in the PRS calculation. DEG analyses were

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215 then carried out as previously described using cell type level expression data, with the trait of
216 interest being MCP-PRS value for each donor rather than chronic pain.
217

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218 **Results**

219 **Chronic pain impacts the brain transcriptome in a region- and cell-type specific manner**

220 In order to identify genes associated with chronic pain in cortical (dACC, DLPFC) and amygdala
221 (medial and basolateral) brain regions, we performed a differential expression analysis
222 comparing bulk tissue from individuals with and without histories of chronic pain. Two genes
223 were significantly downregulated in the dACC in individuals with chronic pain compared to
224 controls (**Figure 1A**), B4GALT2 ($p=1.45x10^{-6}$, $\beta=-0.936$), and VEGFB ($p=1.2x10^{-7}$, $\beta=-$
225 0.945).
226

227 Since bulk tissue gene expression likely represents a combination of signal across cell types
228 sampled in each region, we repeated DEG analyses for each brain region using deconvoluted,
229 imputed cell type level expression data, across six cell types (**Figure 1B**; astrocytes, endothelial
230 cells, excitatory neurons, inhibitory neurons, microglia, and oligodendrocytes). 2093 unique
231 genes were significantly associated with chronic pain in these imputed cell types, with the
232 majority of associations (1810/2326) in BLA microglia of the (**Figure 1C, Figure S1**). Given the
233 relatively small proportions of microglia in these brain regions, these associations represent a
234 significant enrichment over what might be expected by chance ($p_{binomial} < 1x10^{-50}$). There was
235 also a significant enrichment of associations in medial amygdala endothelial cells (N DEGs =
236 254, $p_{binomial} = 1.3x10^{-42}$). Findings in amygdala region oligodendrocyte progenitor cells (OPCs)
237 are presented in the Supplement (**Table S2**).
238

239 **Chronic pain associated genes are enriched in hypoxia response, ribosome component, and**
240 **immunity and infection-related pathways**

241 We explored potential functional impact of chronic pain DEGs through gene set enrichment
242 analyses, including all cell type DEG results with a sufficient number of unique significant
243 genes; dACC microglia, dACC oligodendrocytes, BLA microglia, MeA microglia, MeA
244 astrocytes, MeA endothelial, and MeA oligodendrocytes. Chronic pain associated genes in across
245 these tissues and cell types were enriched for a total of 140 pathway enrichments comprising 101
246 unique pathways ($p_{Bonferroni} = 1.53x10^{-6}$, adjusting across cell and tissue types, **Table S3,**
247 **Supplement**).
248

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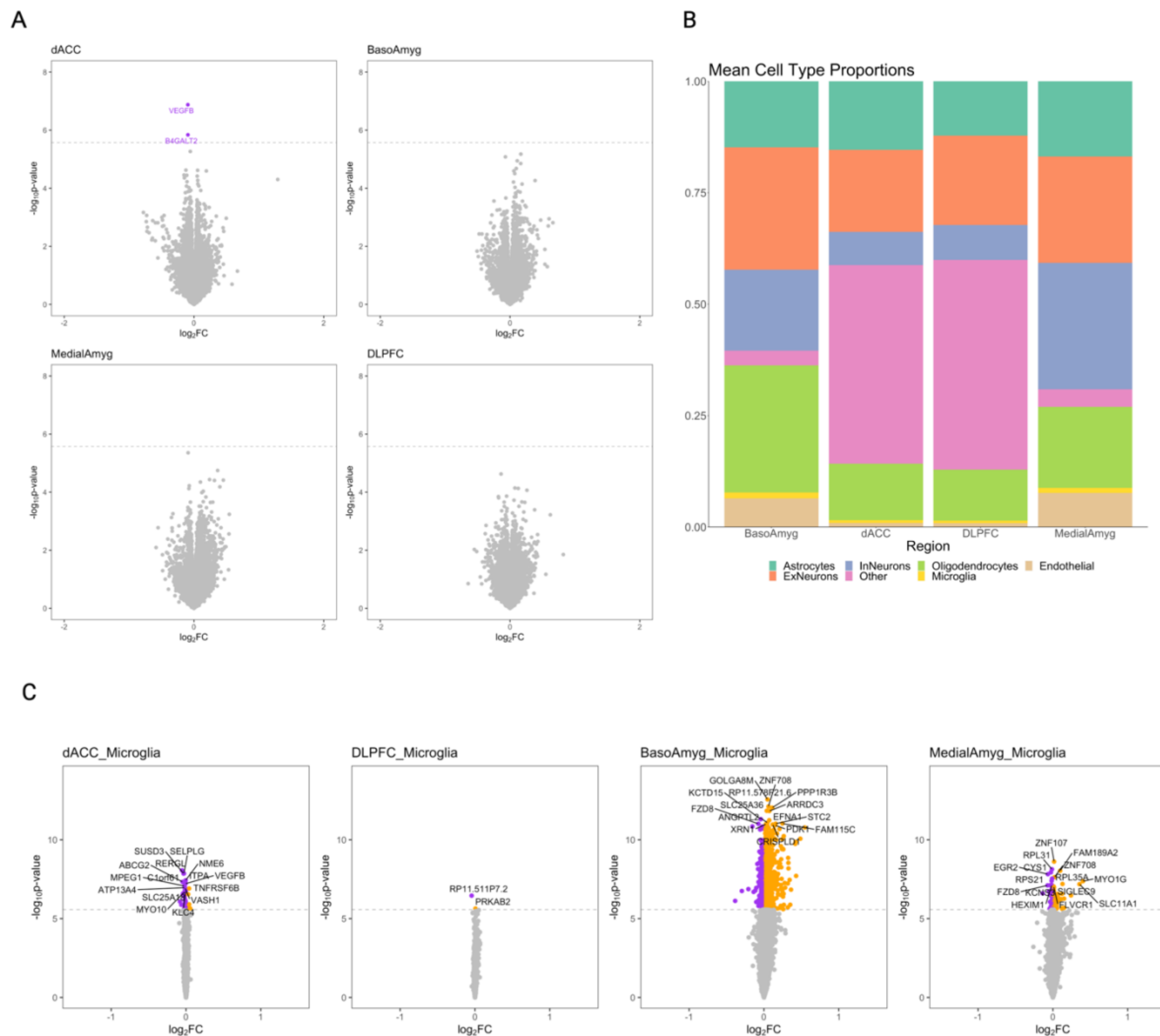

249

250

251 **Figure 1: Chronic pain DEGs are found in the dACC and in microglia of the basolateral amygdala in bulk**
252 **and cell-type-level analyses.**
253 1A: Bulk tissue chronic pain DEGs. Purple: significantly ($P_{Bonferroni} < 0.05$) downregulated, dotted line = DEG
254 regression p value significance threshold for that bulk region. FC = fold change. 1B: Imputed cell type proportions
255 vary across bulk regions – note neurons only present in reference data for cortex (Psychencode) and oligodendrocyte
256 progenitor cells (OPCs) only present in amygdala reference data (Yu et al), OPCs and Neurons both marked 'Other'
257 in this figure panel. FC = fold change. 1C: Chronic pain cell type DEGs in microglia per region. Purple =
258 significantly ($P_{Bonferroni} < 0.05$) downregulated, orange = significantly ($P_{Bonferroni} < 0.05$) upregulated, dotted line = p
259 value significance threshold. For legibility only the top 15 DEGs are labeled in cell type results panel. FC = fold
260 change. InN = inhibitory neuron, ExN = excitatory neuron.

261

262

263 **Significant enrichment of chronic pain genes in transcriptomic experiment results for**
264 **mouse CNS but not human whole blood**

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265 Chronic pain genes differentially expressed in the MeA microglia were significantly (Fisher's p =
266 0.01) enriched for mouse spinal ganglia genes from the McGill TSPdb (**Table 2**). Chronic pain
267 genes DE in the MeA astrocytes, and in dACC microglia and endothelial cells were also
268 significantly enriched for mouse microglia pain genes. There was no significant enrichment of
269 mouse sciatic nerve or mouse brain stem genes in our chronic pain DEG findings.
270
271 Sixty-seven genes identified as significantly DE in microglia were also up/down-regulated in
272 DAMs and/or ARMs (**Table 3**). Of these, 13 show concordant direction of effect across chronic
273 pain, DAMs, and ARMs, 21 show concordant effect across chronic pain and DAMs, and 17
274 across chronic pain and ARMs. None of our chronic pain genes were enriched for pain results
275 from human whole blood (Fisher's $p > 0.05$).
276
277 **Chronic pain and polygenic risk for Multisite Chronic Pain are associated with differential**
278 **expression in unique sets of genes**
279 To assess whether chronic pain DEGs represent predisposition to chronic pain, or are associated
280 with downstream impacts of pain on brain gene expression, we sought to identify DEGs
281 associated with polygenic risk for multisite chronic pain (MCP) at the cell type level. We found
282 21 DEGs significantly associated with MCP-PRS (**Table 4**), none of which were identified as
283 chronic pain DEGs in our previous analyses.
284
285 **Migraine and chronic pain DEGs significantly overlap in medial amygdala endothelial cells**
286 In order to distinguish genes associated with chronic pain from genes associated with specific
287 chronic pain conditions, we tested for migraine DEGs in bulk- and imputed cell type data.
288 Although no genes were significantly associated with migraine in bulk tissue, 942 DEGs were
289 associated at the cell-type level, with an over-representation in microglia (754/942 DEGs,
290 $p_{binomial} < 2x10^{-16}$; albeit in the MeA rather than BLA), in dACC oligodendrocytes (52/942,
291 binomial p = 0.027), and in MeA endothelial cells (117/942, binomial $p = 3.3x10^{-25}$). Twelve
292 DEGs were associated with both migraine and chronic pain (Table S4), representing a significant
293 overlap overall (Fisher's p = 0.004), with this enrichment being driven solely by DEGs in MeA
294 endothelial cells (hypergeometric p = 0.02, Table S5).
295

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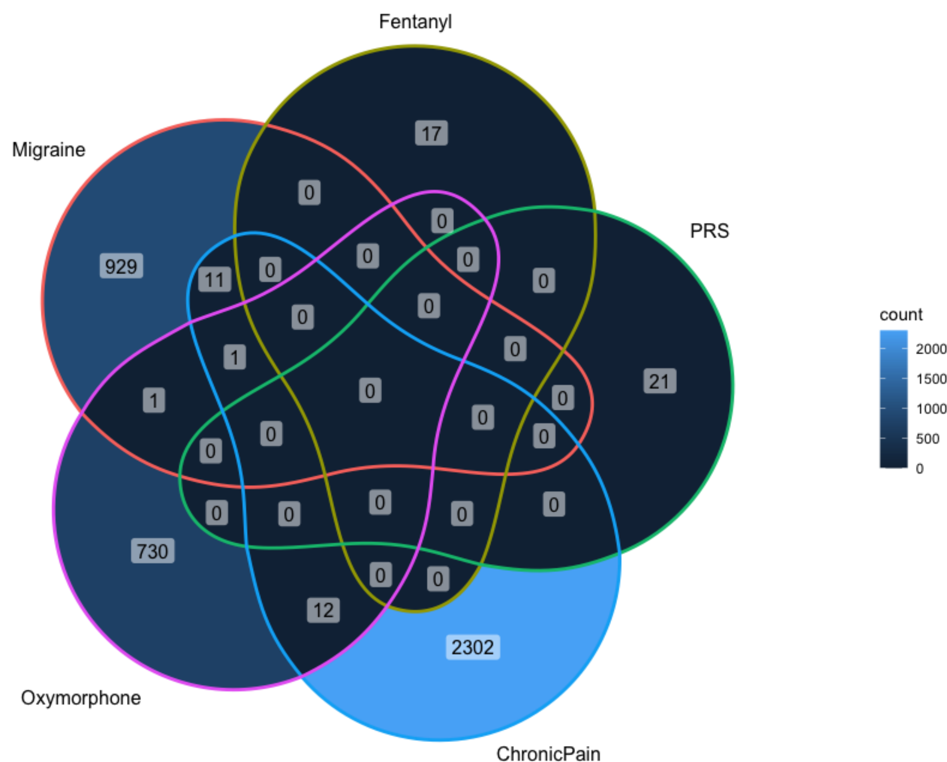
296 **Chronic pain associations are not driven by opioid use**

297 Chronic pain gene associations may also be confounded by long-term use of medications taken
298 to address pain. To test this, we identified DEGs associated with lifetime fentanyl (fentanyl
299 DEGs) and oxymorphone use (oxymorphone DEGs), and compared both single-gene and
300 genome-wide associations to our chronic pain signatures.

301

302 At the bulk level, we identified one gene significantly downregulated in individuals with a
303 lifetime history of oxymorphone use in MeA (GPR158.AS1; $p=1.17\times10^{-06}$). This DEG was not a
304 chronic pain DEG in bulk tissue. At the cell-type level, we identified 744 significant
305 oxymorphone DEGs, of which 13 were also previously associated with chronic pain (Table S6,
306 Figure 2). Oxymorphone DEGs significantly overlapped with chronic pain DEGs
307 (hypergeometric $p < 0.05$) in both BLA and MeA endothelial cells and microglia (Table S7).
308 No genes were significantly associated with fentanyl use in bulk tissue. Cell-type specific
309 analyses identified 17 significant fentanyl DEGs (Table S8); however, none of these DEGs
310 overlapped with chronic pain.

311



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313 **Figure 2: Cell type DEG overlap across all traits (lifetime oxymorphone use, lifetime**
314 **fentanyl use, multisite chronic pain PRS, chronic pain, and migraine) tends to be low.** PRS
315 – polygenic risk score (MCP-PRS).
316
317 **Discussion**
318 Understanding the etiopathology of chronic pain requires careful dissection of chronic pain risk,
319 experience, and treatment. Here, we characterize the impact of each of these distinct factors on
320 brain gene expression; we show that chronic pain primarily impacts gene expression in amygdala
321 microglia, and that this transcriptomic signature is for the most part distinct from the signatures
322 of pain predisposition, or the impacts of taking pain-alleviating medications. Our study is the
323 largest to examine the impact of chronic pain across brain regions through differential expression
324 analysis of brain tissue. Strengths of our study include large sample size, with 304 donors and 4
325 brain regions (all previously implicated in chronic pain) sampled per donor. In addition, a large
326 amount of phenotypic data for each donor is available (160+ variables on lifestyle, trauma,
327 psychiatric and medical diagnoses, and lifetime substance and medication use), allowing us to
328 thoroughly account for sources of variance in gene expression that are not due to chronic pain or
329 other trait-of-interest status. We use two separate region matched reference datasets for cortex
330 and amygdala to study cell type level gene expression imputation. In addition, unusually for
331 chronic pain studies, our sample is almost perfectly balanced in terms of reported sex in donors
332 with chronic pain.
333
334 **Microglia represent a key cell type in chronic pain**
335 We leverage advances in cell-type imputation, coupled with large, well-powered cell imputation
336 reference panels to expand our study beyond region specificity, to look at cell-type specificity.
337 We find the majority of our associations in microglia. In addition, we find our chronic pain genes
338 to be enriched in mouse results in CNS and microglia from the pain transcriptomic signatures
339 database, and to show partial overlap with genes associated with DAMs (disease-activated
340 microglia) and ARMs (activated response microglia), reactive microglia phenotypes associated
341 with neurodegenerative disease [46,51,52].
342

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343 In the context of chronic pain, microglia have previously been implicated in imaging studies of
344 low back pain patients [53], in the transition from acute to chronic pain in rodent models of spinal
345 nerve transection [54], in chronic visceral pain [55], and in general neuroplasticity related to chronic
346 pain [56]. Specifically in BLA microglia, studies in rodents found inhibition of these cells increased
347 anti-nociceptive effects of opioid drugs [57], and reduced pain-induced depressive behaviour in a
348 bone cancer pain model [58]. Our results indicate that BLA microglia play an important part in
349 human chronic pain, that significant overlap is present between human and mouse chronic pain
350 DEGs in microglia, and that the transcriptomic changes in microglia in chronic pain do not
351 exactly match many of those seen in DAMs/ARMs.
352
353 **Impact of pain-related factors and conditions on gene expression in the brain**
354 Importantly, our study compares and contrasts different factors involved in pain. There may be
355 key differences in transcriptional changes that occur as a result of living with chronic pain, with
356 predisposition to developing chronic pain, and with indirect results of chronic pain such as
357 potential opioid use. Furthermore, distinct genes may be differentially expressed in chronic pain
358 compared to more specific chronic pain conditions such as migraine.
359
360 **Genetic predisposition to chronic pain is transcriptomically distinct from experience of**
361 **chronic pain**
362 We found distinct sets of genes associated with experience of chronic pain (i.e., observed in brain
363 expression) compared to genetic predisposition to chronic pain (which we measure through
364 polygenic risk for Multisite Chronic Pain). We note that although our sample size (304 donors) is
365 low for polygenic risk score analyses, this sample size paired with the large base GWAS (N >
366 380, 000 participants), is considered acceptably powered [59].
367
368 There are no DEGs shared between polygenic risk for multisite chronic pain and chronic pain as
369 a trait. In contrast to chronic pain DEGs, DEGs for polygenic risk of multisite chronic pain were
370 found primarily in dACC oligodendrocytes (19/21 DEGs), in contrast to BLA microglia which
371 appear to play a role in established chronic pain. Previous studies have shown oligodendrocytes
372 may contribute to chronic pain development [60], in addition to evidence in rodent models that
373 oligodendrocyte ablation can induce central pain without immune / other glial cell input [61].

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374

375 Genes differentially expressed with increasing polygenic risk of MCP included genes with
376 previous evidence of involvement in pain and cardiac phenotypes, including Fam13b (associated
377 with mouse cardiac phenotypes, 82), TRAF5 (vascular inflammation and atherosclerosis, 83),
378 and FAM184B (heart disease and hip pain [64]). These associations are in line with established
379 links between chronic pain and heart conditions [65], including in our own previous work showing
380 pain gene associations with cardiac dysrhythmias in a large EHR [14]. Other MCP-PRS associated
381 genes have previous evidence of association with pain phenotypes, including DOCK3 [66],
382 LAMB1 [67], DUSP4 [68], and C1QTNF7 [69]. Several genes were associated with nerve injury and
383 neuropathies, including NCOA5 [70], EPHA5 [71], FZD4 [72], and MRPL48 [73], and NFKBID [74].
384

385 **Opioid and chronic pain DEG overlap is small and concentrated in the amygdala**
386 At the cell level, none of the chronic pain DEGs overlapped with those associated with lifetime
387 fentanyl use, and 13 DEGs overlapped between chronic pain and lifetime oxymorphone use.
388 These 13 overlapping DEGs represented significant general enrichment of chronic pain DEGs
389 within oxymorphone DEGs (Fisher's $p = 0.0001$), but this was driven by four specific cell types
390 (each showing hypergeometric $p < 0.05$); BLA microglia, BLA endothelial cells, MeA microglia
391 and MeA endothelial cells.
392

393 Overlapping DEGs in oxymorphone and chronic pain had concordant direction of effect (i.e.,
394 genes significantly downregulated in chronic pain tend to also be downregulated in lifetime
395 oxymorphone use). Although we did not explicitly examine opioid induced hyperalgesia (OIH),
396 these results suggest transcriptomic changes associated with both increased pain and
397 oxymorphone use are present in these cell types of the amygdala, potentially representing
398 relevant genes and cell types in OIH.
399

400 Genes differentially expressed in both oxymorphone and chronic pain (13 DEGs) included those
401 implicated in TNF response and previously linked to Lyme disease (YBX3, [75,76]), encoding
402 cytokines (CCL2, [77]), Raine syndrome and bone mineralization (FAM20C, [78,79]), autocrine
403 signaling and lipid storage (HILPDA, [80]), connective tissue biogenesis (LOXL2, [81]),
404 neurogenesis (RASF10, [82]), and Alzheimer disease, Parkinson disease, schizophrenia, and PTSD

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405 (SERPINA3, [83–86]). YBX3 gene expression in the brain has also been previously linked to
406 PTSD[87].
407
408 **Migraine and chronic pain DEG overlap is small and driven by amygdala endothelial cells**
409 Pathophysiology of migraine is not completely understood [88,89], and as with all conditions where
410 chronic pain is a main symptom, there may be distinct mechanisms underlying condition-specific
411 processes and potential tissue damage vs a general chronic pain phenotype. We explored this
412 through comparing genes differentially expressed in migraine and in chronic pain, finding small
413 (12 DEGs) but significant overlap driven by endothelial cells of the medial amygdala.
414
415 Endothelial cells interface with vasculature, and endothelial dysfunction has been previously
416 implicated in migraine, although it is not fully established whether this dysfunction is a cause or
417 consequence of migraine [90]. Significant overlap in chronic pain and migraine DEGs in medial
418 amygdala endothelial cells could therefore mean transcriptomic changes at endothelial cells and
419 migraine-related pain are specifically linked (as opposed to non-pain related migraine symptoms,
420 and non-migraine/ general chronic pain).
421
422 In addition, VEGF, encoded by VEGFA, is a 'protective angiogenic factor' in migraine [90].
423 VEGFB (in contrast to VEGFA) is 'dispensable' in new blood vessel growth in normal tissue but
424 seems to play a more essential role in neurodegenerative diseases and stroke (protecting various
425 vasculature during disease progression) [91]. VEGF has also been implicated in rodent and human
426 studies of neuroplasticity, stress response, and antidepressant action [92–96]. Our results showed
427 VEGFB was downregulated in bulk dACC tissue in chronic pain, indicating VEGFB may
428 represent a protective angiogenic factor outside of migraine, and in a general chronic pain
429 context.
430
431 **Limitations**
432 There are some key limitations of our study that should be borne in mind when interpreting our
433 results. The chronic pain phenotype is extremely broad and dichotomized. Opioid use
434 phenotypes were similarly broad, and lifetime use did not delineate between prescribed/ non-
435 prescribed, and current or past use. When comparing lifetime fentanyl use and lifetime

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436 oxymorphone use, 'case' numbers are higher for fentanyl, but far fewer DEGs are found – there
437 are also no overlapping DEGs between the two traits. This may reflect different clinical uses of
438 these drugs, with fentanyl as a more discrete rather than chronic exposure, making effects on
439 gene expression more difficult to capture post-mortem and potentially long after a brief
440 exposure.
441 Another limitation is lack of human brain tissue experiment data available in the McGill TSPdb
442 for direct comparisons with our results, although we explored enrichment in human whole blood
443 and mouse brain and nervous system results.
444
445 **Conclusions**
446 Our results suggest that chronic pain impacts gene expression in a heterogeneous way within
447 brain regions depending on cell type, and signal from cell type expression changes may be
448 hidden in bulk tissue analyses due to heterogeneous cell type populations. We highlight brain
449 region and cell type specific genes differentially expressed in chronic pain. In particular, our
450 results suggest BLA microglia are a key cell type in chronic pain, and that pain related
451 transcriptomic changes are distinct from those seen in neurodegenerative disease and general
452 neuroinflammation. Our microglia findings were also enriched for genes differentially expressed
453 in mouse microglia and spinal ganglia samples, but not in human whole blood, again
454 emphasizing region and cell specificity and suggesting a degree of cross-species conservation of
455 certain chronic pain associated transcriptomic changes. Pathways enriched for genes
456 significantly differentially expressed in these cells in chronic pain include those involved in
457 immune processes and hypoxia response. Migraine, opioid use, genetic predisposition to chronic
458 pain, and chronic pain are all largely distinct at the transcriptome level. Points of overlap, though
459 overall consisting of a small number of genes, can inform potential mechanisms underlying
460 shared characteristics between traits. Differences at the transcriptome level between
461 predisposition to chronic pain and chronic pain can be potentially used to inform tailoring of
462 treatment to stage in chronic pain development.
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